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# EFFECT OF BLOOD TRANSFUSIONS ON IMMUNE FUNCTION. VII. IDENTIFICATION OF BLOOD TRANSFUSION-INDUCED SUPPRESSOR CELL

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Blood transfusions have previously been shown to impair immune function and to increase susceptibility to infectious complications. To determine the mechanism of this immunosuppression, adult Lewis rats were administered syngeneic blood, allogeneic blood, or saline. One week later, splenic lymphocytes and peritoneal macrophages were harvested from these animals and injected intravenously into other Lewis rats. These rats were then challenged with intraperitoneal *Escherichia coli*. The allogeneically transfused rats had a decreased survival rate which approached significance ($p = 0.055$). When an identically obtained set of macrophages and lymphocytes were added to mixed lymphocyte reaction cultures, the macrophages obtained from allogeneically transfused rats were found to significantly impair the lymphocyte's blastogenic response ($p < 0.05$). In conclusion, at one week following transfusion, the macrophage appears to be the cell which mediates the posttransfusion immunosuppressive response.

KEY WORDS: Transfusions, immunosuppression, macrophages.



Prior to the 1970s, it was believed that the administration of blood transfusions would result in a slight immunostimulatory effect on patients. This belief was based primarily on two observations, the first being the widely recognized effect of transfusing incompatible red cells into recipients and the strong immunologic reaction which resulted in massive red blood cell lysis.[1] The second was the demonstration by Medawar that the parenteral administration of blood products from a specific donor resulted in an immunization of the recipient that resulted in a more rapid rejection of skin allografts harvested from the same donor.[2,3]

This belief led surgeons to avoid administering blood transfusions to patients who were awaiting solid organ transplantation. This policy changed in the early 1970s when a number of animal and human studies demonstrated that transfusions administered prior to transplantation decreased the rate of allograft rejection.[4–8]

In the 1980s, there have been a number of studies evaluating the effect of perioperative blood transfusions on tumor growth and metastases in patients undergoing

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oncologic procedures.[9] These reports dealt with colon,[10–15] lung,[16,17] and mammary carcinomas[18–21] and sarcomas.[22] Nine of these reported an increased incidence of tumor recurrence and a diminished long-term survival rate in those patients receiving perioperative transfusions.[10–13,16–19,22] The remaining four failed to demonstrate any effect on long-term survival in patients receiving perioperative transfusions.[14,15,20,21]

It has more recently been demonstrated in a number of animal studies that blood transfusions increase susceptibility to infectious complications.[23,24] It has also been documented in a retrospective study of patients undergoing colon cancer procedures that perioperative transfusions have a strong correlation with postoperative infectious complications.[25]

Herein we attempt to identify the suppressor cell generated by blood transfusions that causes the increased susceptibility to infectious complications.

## METHODS

### *Animals*

Adult Lewis rats weighing $\pm 250$ g were used for all phases of this study. Adult male A'Sogaloff Cancer Institute (ACI) and Lewis rats were used as blood donors. All animals were housed in stainless steel cages and were given food and water *ad libitum*. The animals were observed for a minimum of 1 week prior to entry into the study to check for any evidence of preexisting disease.

### *Transfusions*

Blood was obtained from donor animals by cardiac puncture. The blood was mixed at a 4:1 volumes ratio with CPDA-1 standard anticoagulant and stored at 4 °C for 1 day prior to use. The transfusions were given intravenously at a dosage of 0.5 ml per animal.

### *Controls*

Animals in the control group received 1.5 ml normal saline intranvenously. The increased volume of saline was chosen since it was believed that this would most closely approximate the intravascular volume changes seen with the administration of 0.5 ml whole blood used in the transfusion groups.

### *Bacteria*

The *Escherichia coli* strain was generously supplied by Dr. Richard Simmons' laboratory. It was initially obtained from a human infection at the University of Minnesota Hospital and has been demonstrated to cause a lethal peritoneal infection in a standard rat model.[26] The *E. coli* was incubated for 18 hr at 37 °C in a standard trypticase soy broth and then washed 3 times in normal saline to remove free endotoxin. Appropriate dilutions were made with normal saline to achieve a final concentration of $1 \times 10^7$ cfu/ml of normal saline. Prior incubations had determined the amount of saline necessary to achieve this dilution. One ml of this suspension was injected intraperitoneally through a 22-ga needle for the septic phases of this study.

*Cell Transfer Procedures*

To determine the recipient cell responsible for the posttransfusion immunosuppression, Lewis rats were divided into 3 groups. One group ($n = 60$) received 1.5 ml saline intravenously, the second group ($n = 60$) received 0.5 ml syngeneic Lewis blood, and the final group ($n = 60$) received 0.5 ml allogeneic ACI rat blood. These rats were followed for 1 week prior to sacrifice.

Following sacrifice, the peritoneal cavities were entered in an aseptic manner. The perintoneal cavities were lavaged with 20 ml of HBSS without calcium or magnesium and with 10 mM EDTA.

Simultaneously, the spleens were removed and bivalved. Splenocytes were harvested by injecting HBSS into the splenic pulp and collecting the effluent. The splenocyte suspensions were cultured for 1 hr in flat-bottom culture plates to allow for adherence of any monocytes/macrophages. The lymphocyte-rich suspensions were then removed.

Both the periotoneal macrophage and splenic lymphocyte suspensions were hypotonically lysed of any contaminating red blood cells. The suspensions were washed 3 times with HBSS containing calcium and magnesium. The number of macrophages and lymphocytes per suspension were determined using Wright's stain and a hemocytometer. The lymphocyte and macrophage suspensions were then diluted in sufficient HBSS to achieve a final concentration of $2 \times 10^8$ cells/ml for use in the *in vivo* septic phase of the study.

These cell suspensions were injected intravenously into previously untreated Lewis rats. There were 60 rats used in each of the 6 treatment groups (splenocytes from saline, allogeneic and syngeneic transfused rats plus macrophages from saline, allogeneic, and syngeneic transfused rats). One hr later, the same rats were injected with $1 \times 10^7$ of the *E. coli* suspension intraperitoneally. These rats were observed for 7 days following the bacterial challenge to determine mean survival times and absolute survival rates.

*In vitro Assays*

The splenic lymphocytes and peritoneal macrophages previously described were also assayed for their ability to inhibit a mixed lymphocyte reaction (MLR). For this assay, splenocytes were harvested from healthy untreated adult male Buffalo (BUF) and Lewis rats. The splenocytes were purified as previously described. Splenocytes obtained from the BUF rats were treated with mitomycin to prevent these cells from undergoing a blastogenic response. $1 \times 10^5$ BUF rat splenocytes were incubated with $1 \times 10^5$ Lewis rat splenocytes in standard RPMI media. To these cultures was added $1 \times 10^4$ of either the peritoneal macrophages or splenic lymphocytes obtained from allogeneically transfused rats ($n = 10$), syngeneically transfused rats ($n = 10$), and saline-treated rats ($n = 10$). The MLR cultures were incubated for 4 days at 37 °C in a 5% $CO_2$ atmosphere. At the end of this time, they were pulsed with 0.5 $\mu$C tritiated thymidine. Eighteen hr later, the cultures were harvested and passed through cell filters. The filters were placed in standard liquid scintillation vials to which was added scintillation fluid and they were counted on a beta particle scintillation counter.

The splenic lymphocytes were also assayed for the presence of pan (OX-19), helper/inducer (W3/25), and suppressor/cytotoxic T cell (OX-8) receptors. These assays were performed using standard monoclonal antibody preparations and fluo-

rescent-labelled anti-mouse IgG as the second-step antibody. Fluorescent-labelled cells were then analyzed using standard flow cytometry.

### *Statistical Analysis*

Data were analyzed for statistical significance using ANOVA and the Newman-Keuls multiple range test. Mean survival times were analyzed using the Wilcoxon test and survival rates with Chi square.

## RESULTS

The addition of lymphocytes from syngeneically and allogeneically transfused rats to the MLR had no significant effect on the blastogenic response (Table I). The addition of macrophages from the transfused rats did exert a significant inhibitory effect on the blastogenic response of the MLR (Table I).

The percentages of T cell subsets in each of the three treatment groups are shown in Table II. There were no significant differences among any of these groups.

In the *in vivo* septic phase of the study, there was no effect noted with the addition of lymphocytes from any of the three treatment groups. The survival rate of the animals receiving lymphocytes from saline-treated animals was 67%. Those animals receiving lymphocytes from syngeneically transfused rats had a 64% survival rate as did the rats who received lymphocytes from allogeneically transfused rats. The mean survival times for these groups were 5.49 $\pm$ 0.35 days for rats receiving lymphocytes from saline-treated rats, 5.22 $\pm$ 0.38 days for rats receiving lymphocytes from syngeneically transfused rats, and 5.29 $\pm$ 0.37 days when the lymphocytes were obtained from allogeneically transfused rats. None of these differences achieved statistical significance.

The mean survival times for rats infused with macrophages prior to *E. coli* challenge were 5.64 $\pm$ 0.35 days when the macrophages were obtained from saline-treated animals, 5.00 $\pm$ 0.37 days when the macrophages were obtained from syngeneically transfused rats, and 5.01 $\pm$ 0.38 days when macrophages were obtained from allogeneically transfused rats. These differences were not statistically significant. The survival rate of rats that received macrophages from saline-treated rats immediately prior to bacterial challenge was 73%. When the injected macrophages were obtained from syngeneically transfused rats, this survival rate decreased to 56%. Administration of macrophages obtained from allogeneically transfused rats decreased the survival rate to 53%. These differences approached statistical significance ($p = 0.055$).

TABLE I

Mixed lymphocyte reaction results (cpm) with addition of either lymphocytes or macrophages from transfused rats

| Group | Saline | Syngeneic | Allogeneic |
|---|---|---|---|
| Lymphocytes | 6546 $\pm$ 885 | 6632 $\pm$ 826 | 9261 $\pm$ 1117 |
| Macrophages* | 1612 $\pm$ 336 | 1141 $\pm$ 199 | 825 $\pm$ 172 |

*$p < 0.05$.

TABLE II
Percentages of T lymphocyte subsets in rats administered transfusions

| T lymphocytes | Saline group | Syngeneic group | Allogeneic group |
|---|---|---|---|
| Pan (OX-19) | 65.2 ± 5.0 | 74.5 ± 3.8 | 70.1 ± 4.2 |
| Helper/Inducer (W3/25) | 53.2 ± 1.9 | 58.8 ± 1.0 | 56.2 ± 2.0 |
| Suppressor/Cytotoxic (OX-8) | 16.2 ± 0.3 | 16.8 ± 0.5 | 16.8 ± 0.5 |

## DISCUSSION

The modern era of blood transfusion began in 1900 with Landsteiner's demonstration of three of the four main blood groups.[27] This has allowed surgeons and physicians to use transfusions to safely correct severe anemias and significant blood losses resulting from surgery or traumatic injuries. Until recently, there has been little investigation on the immunologic sequelae of such transfusions.[9]

During the past two decades, it has been shown that transfusions increase allograft survival in transplant recipients by suppression of the normal immune function.[4-8] This posttransfusion immunosuppression was originally thought to be of a narrow spectrum directed primarily towards preventing a host response to allograft antigens. This view has been brought into question by a number of recent studies. These studies have demonstrated that perioperative blood transfusions are associated with an increase in the rate of tumor growth and metastasis in oncologic patients.[9]

There have also been a number of studies investigating the effects of blood transfusions on immune function in nonsurgical patients. These have shown that patients receiving transfusions have a decrease in their OKT4/OKT8 (helper/suppressor) T-lymphocyte ratio.[8,30] Natural killer cell function has also been documented to show a significant impairment following transfusion.[30] Finally, Watson *et al.*[31] has demonstrated a fall in cell-mediated immune function, as measured by skin response to the hapten DNCB following transfusion. A major weakness in all of these studies is the presence of a serious underlying disease process in all of the patients which necessitated the transfusions. The possibility that this underlying disease process altered the immunologic function assayed cannot be excluded.

To eliminate this possibility, we have previously reported on a series of animal studies which demonstrated a number of similar immunologic alterations following allogeneic blood transfusions. Among these alterations are a decreased cell-mediated immune response, similar to that seen by Watson *et al.*, as measured by hapten sensitization to DNFB.[32] Alterations documented in macrophage function included a decreased peritoneal migration of macrophages in response to a chemical peritonitis.[32] These macrophages were further shown to have alterations in the rate of production of arachidonic acid metabolites.[33] These included a 50% increase in the rate of production of prostacyclin and thromboxane and a 150% increase in the rate of production of prostaglandin E. This increase in prostaglandin E production was also noted in traumatized rats that received allogeneic transfusions.[34] The transfusions failed to alter the rate of production of the immunostimulatory leukotrienes. Allogeneic transfusions were shown to impair the local response to bacterial challenge.[35] The administration of allogeneic blood increased the rate of tumor growth in a Fischer rat sarcoma model[36] and in a Wistar-Furth metastatic colon cancer model.[37] Allogeneic transfusions have also been demonstrated to decrease survival rates in

multiple septic animal models. These models have included burned rats challenged with *Pseudomanas aeruginosa* (Strain 1244)[23,28] and unburned rats challenged with intraperitoneal *E. coli*.[24]

Support for the concept that transfusions increase susceptibility to bacterial infections also comes from the work of Tartter *et al.*[25] who, in a retrospective study of surgical patients with colon cancer, showed an increased infection rate in patients receiving perioperative transfusions as compared to nontransfused patients. This transfusion effect was present even after considering the patient's age, length of surgery, or extent of surgical resection.

The purpose of our current study was to identify the immunosuppressive cell resulting from blood transfusions which might lead to increased susceptibility to bacterial infections. This was attempted using both *in vitro* and *in vivo* models.

In the *in vitro* (MLR) models, the lymphocytes were unable to significantly alter the blastogeneic response and failed to demonstrate any significant differences in T cell subsets among the three treatment groups. In the *in vivo* (septic animal) model, the lymphocytes failed to alter either the mean survival time or the absolute survival rate. This would appear to indicate that any immunosuppression seen at 1 week following transfusion is not due to primary alterations in lymphocyte function.

The macrophages demonstrated an ability to alter immune function. The fact that the macrophage, and not the lymphocytes, was found to be immunosuppressive in our models is somewhat surprising in the light of prior laboratory transplant studies. In most of these studies, the decreased rejection rate seen following transfusion was shown to be a result of increased suppressor T-lymphocyte function.[9] These studies, however, generally waited for more than 1 week prior to assaying immune function. It therefore may be that the initial cellular alteration following transfusion is an enhancement of suppressor macrophage function, resulting in increased production of immunosuppressive macrophage products such as prostaglandin E. The increased production of prostaglandin E might then increase suppressor lymphocyte function over a more prolonged period of time.[39]

If this is indeed the mechanism of the posttransfusion immunosuppression, then prevention of this immunosuppression might be possible by the simultaneous administration of drugs which block macrophage secretion of immunosuppressive arachidonic acid metabolites. One such drug, indomethacin, has been shown to be able to at least partially block the transfusion effect in a rat heart transplant model.[40]

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